United States Department of Agriculture
NRCS Natural Resources Conservation Service
TECHNICAL NOTES

Agronomy WY-3 January, 2003

Subject: Nitrogen Fixation and Legume Inoculation

I. INTRODUCTION

By volume, about 79% of the air we breathe are nitrogen. This nitrogen is in a free or uncombined state. Approximately 35,000 tons of this free nitrogen occurs over every acre of land. Nitrogen in this free state is useless to plants unless it can be combined with other elements. This conversion can be made certain bacteria living in the soil in association with leguminous plants. This source of nitrogen can be utilized for insurance of a successful stand, soil improvement, higher yields and improved crop quality by inoculating legume seeds with the proper bacteria at the time of seeding.

II. LEGUMES

There are more than 12,000 species of legumes and of these about 50 species are grown commercially in the United States. In Wyoming, legumes are one of our most efficient and economical sources of winter protein for livestock. Legume provides phosphorus, calcium, vitamins and high quality protein. The protein content is directly related to the high nitrogen content.

III. LEGUME BACTERIA

Life in the soil is quite diverse, ranging from microscopic single-celled organisms to large burrowing animals. Attachment #1 provides a general outline of plant and animal life found in the soil.

Bacteria are single-celled plants and exceed all other soil organisms in numbers and kind. There may be over one ton of bacteria in an acre of soil. Most bacteria are rod-shaped, approximately one micron in diameter, and up to two or three microns in length (1 micron = 1/25,000 of an inch). Based on their energy requirements, soil bacteria can be divided into two large groups. Bacteria which obtain their energy from oxidation of inorganic elements or compounds, their carbon from carbon dioxide and their nitrogen from inorganic compounds are autotrophic bacteria. Heterotrophic bacteria obtain their energy and carbon from complex organic substances. These heterotrophic bacteria are further divided into those requiring fixed nitrogen and those utilizing free nitrogen or the nitrogen

fixers. Nitrogen fixing bacteria are either nonsymbiotic or sybiotic. It is the sybiotic nitrogen fixing bacteria that we are concerned with in the inoculation of legumes. Bacteria in this group are of genus Rhizobium.

IV. NITROGEN FIXATION PROCESS

In the process of sybiotic nitrogen fixation, the Rhizobium bacteria in the soil make contact with the root hairs of the legume plant. This causes the root hairs to curl. An infection thread is formed on the root through which the bacteria migrate to the center of the root. Inside the root the bacteria migrate to the center of the root. Inside the root the bacteria multiply and form bodies called bacteroids. The enlargement that occurs in the root as a result of this growth is the nodule that we see on the roots of legumes (Attachment #2). The bacteroid receives nitrogen from the bacteria that has been fixed from the free nitrogen in the air. The host plant can also benefit from increased eater uptake and nutrient absorption due to the increased surface area of the root caused by the nodule. Associated plants are benefited by nitrogen that is excreted from the nodule and when the nodules disintegrate and decompose. The nodules disintegrate rapidly at the time of seed formation.

V. LEGUME NODULES

The absence of nodules on the roots would indicate that the plant derives all of its nitrogen from the soil. Nodules on the root are a sign that the plant may be benefiting from fixed nitrogen.

Nodules that are pink or red inside are active in producing nitrogen for the host plant. If the nodules are white, green, or brown, little or no nitrogen is being fixed. Not all Rhizobium are beneficial in the fixation process. Some forms of Rhizobium bacteria enter the root and form small white nodules but do not fix any nitrogen. It has been estimated that only about 25% of the Rhizobium present are beneficial nitrogen-fixing bacteria are present.

VI. LEGUME INOCULATION

We just remember that bacteria are microscopic one-celled plants. Being plants, they have requirements for temperature, moisture, nutrients, etc., as do higher plant forms. It is for this reason that in Wyoming, native populations of bacteria are low. Extreme cold temperatures in winter, high summer temperatures, low organic matter content, low rainfall, and the drying out of soil after a crop is harvested and irrigation ceases are not conductive to a favorable microbal population. To insure that adequate inoculation occurs, all legume seedings should be inoculated at the time of seeding.

Inoculants can be purchased from the seed supplier at the time the seed is obtained. Legume bacteria are highly selective and not all bacteria will function on all legumes. It is important to obtain a culture of the kind of bacteria for the legume you intend to plant. These different kinds of bacteria are placed into groups called cross-inoculation groups. There are eight main cross-inoculation

groups. These groups and some of the more important legumes in each group are listed in Attachment #3.

Inoculant is usually sold in one of three types of carriers. These carriers are (1) humus or peat, (2) agar, and (3) liquid. A majority of the carriers are the humus type.

The inoculant is usually mixed with water to form a tin paste and the mixture poured over the seed and thoroughly mixed to coat all of the seed. The use of water will reduce the amount of inoculant needed for a given amount of seed. Sometimes sugar or milk is added to make the mixture more adhesives. Always read and follow the directions on the package to insure a successful inoculation of legume seeds.

When working with an inoculant, some of the important items to remember are

(1) Use a viable inoculant for the species of legume you are going to seed.
(2) Mix only the amount of inoculant and seed that you plan to seed at that time.
(3) Protect inoculated seed from high temperature and sunlight.
(4) Plant seeds as soon as possible after inoculation.

VII. PRE-INOCULATED SEED

It is becoming more popular to buy seed that has already been inoculated by the seed producer or seed dealer. If such seed is purchased, remember the basic rules in handling the inoculant. Don't allow the pre-innoculated seed to become exposed to extremes in temperature. Store the seed in a cool, dry place. If you have any doubts as to the viability of the inoculant or the seed is not planted as scheduled – RE-INOCULATE.

VIII. CHEMICAL SEED TREATMENTS

Inoculation of legume seed should not be confused with chemical seed treatments. Chemical seed treatments are applied to control fungi, insects and injurious soil organisms. Most seed disinfectants are toxic to legume bacteria. Read the label of any chemical seed treatment product before use.

* CAUTION: If pesticides are handled or applied improperly, or if unused portions are to disposed of safely, they may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife; and they may contaminate water supplies. Drift from aerial spraying can contaminate nearby crops and other vegetation. Follow the directions and heed all precautions on the container label.

IX. FERTILIZERS

Complete fertilizers should not be allowed to come in direct contact with inoculated legume seeds. Phosphates are not as harmful as nitrogen or potassium fertilizers. Normally if the concentration of fertilizers does not injure seed germination, it will not ordinarily harm legume bacteria.

GENERAL OUTLINE OF SOME OF THE PLANT AND ANIMAL LIFE IN THE SOIL

VERTIBRATES: (Gophers, Moles, and Reptiles)
ARTHROPODS: (Woodlice, Spiders, Mites, Millipedes, and Ants)
MOLLUSKS: (Snails, Slugs)
WORMS: (Nematodes, Earthworms)
PROTAZOA: (Amoebae, Ciliates, and Flagellates)
ROOTS OF HIGHER PLANTS: (Trees, Shrubs, and Crops)
ALGAE: (Green, Blue, and Diatoms)
FUNGI: (Molds, Yeast)
ACTINOMYCETES: (Many kinds)
BACTERIA: The simplest form of plants

A. AUTOTROPHIC:

1. Nitrite Formers
2. Nitrate Formers
3. Sulfur Oxidizers
4. Iron Oxidizers
5. Those that act on hydrogen and various hydrogen compounds

B. HETEROTROPHIC:

1. Those requiring fixed nitrogen:
2. Nitrogen fixers:

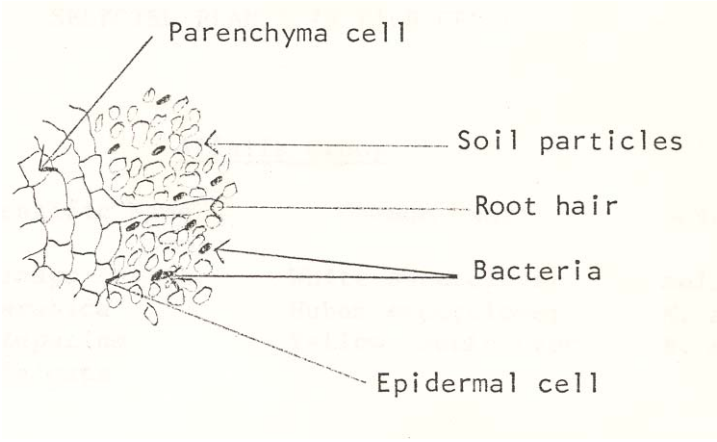
   a. non-symbiotic
   b. Symbiotic

-Bacteria may be aerobic or anaerobic
Aerobic- Require gaseous oxygen
Anaerobic-Do not require gaseous oxygen

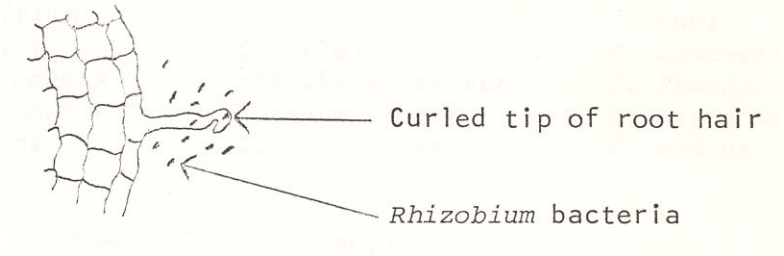
-Bacteria may be autotrophic or heterotrophic
Autotrophic-Utilize carbon dioxide or carbonates as source of carbon and oxidize inorganic (Fe, NH_4, S, NO_2, etc.) elements or compounds as energy source
Heterotrophic- Utilize organic materials to obtain energy and are incapable of utilizing inorganic compounds
-Bacteria may be non-symbiotic or symbiotic
Non-symbiotic – Free living
Symbiotic- Living in association with another organism for mutual benefit

The bacteria associated with nitrogen fixation in legumes are aerobic, heterotrophic, symbiotic organisms.

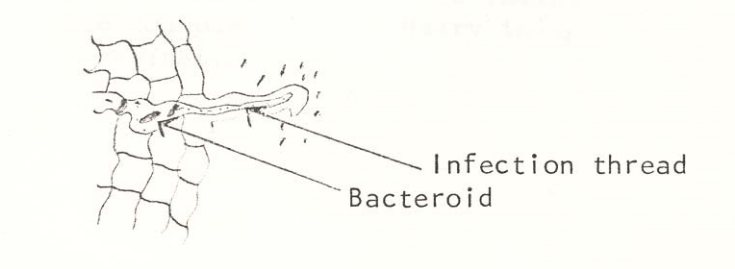
A. Bacteria respond to a product of the host plant and organisms move toward root hair.



B. When bacteria make contact with root hair, the root hair curls.



B. Bacteria enter root hair and an infection thread enters the root. The bacteria migrate through the infection thread to center of root. Inside the root the bacteria rapidly multiply and form irregular swollen bodies called bacteroids receive food from host plant which it uses to fix nitrogen from atmosphere. The host plant benefits from the fixed nitrogen. The enlargement of the bacteroids causes the formation of the nodule.



CROSS INOCULATION GROUPS WITH SOME SELECTED PLANTS IN EACH GROUP

Alfalfa Group

Common Name	Scientific Name	Common Name	Scientific Name
Alfalfa	Medicago Name	White sweetclover	Melilotus alba
Spotted bur-clover	M. arabica	Hubam sweetclover	M. alba annua
Black Medic	M. lupulina	Yellow sweetclover	M. Officinalis
Yellow alfalfa	M. falcata		

Clover Group

Alsike clover	Trifilium hybridum	Ladino clover	T. repens (giganteum)
Hop clover	T. agrarium		
Sibthorp Suckling Clover	T. dubium	Sub clover	T. subterraneum
Schreb Field Clover	T. procumbens	Strawberry clover	T. fragiferum
Red Clover	T. pratense	Berseem clover	T. alexandrinum
Whiteclover	T. repens	Zigzag clover	T. medium

Pea and Vetch Group

Field peas	Pisum arvense	Narrowleaf vetch	V. angustifolia
Garden pea	P. sativum	Purple vetch	V. atropurpurea
Austrian winter pea	P. sativum (var. arvense)	Sweet pea	Lathyrus odoratus
Common vetch	Vicia sativa	Rough pea	L. hirsustus
Hairy vetch	V. villosa		

Cowpea Group

Cowpea	Vigna sinensis	Lima bean	Phaseolus lunatus (macrocarpus)
Common lespedeza	Lespedeza striata		
Korean lespedeza	L. stipulacea	Wild-indigo	Baptisia tinctoria
Alyceclover	Alysicarpus vaginalis	Hairy indigo	Indigofera hirsuta

Bean Group

Common Name	Scientific Name	Common Name	Scientific Name
Garden beans, kidney Bean, navy bean, Pinto bean	Phaseolus vulgarius	Scarlet runner bean	P. coccineus (multiflorus)

Lupine Group

Blue lupine	Lupinus Angustifolius	White lupine	L. albus
		Washington lupine	L. polyphyllus
Yelloe lupine	L. luteus		

Soybean Group

All varieties of soybeans----------Glycine max (Soja max)

Specific Strain Group

Birdsfoot trefoil	Lotus corniculatus	Siberian pea-shrub	Caragana arborescens
Big Trefoil	L. uliginosus		
Sainfoin	Onobrychis viciaefolia	Leadplant	Amorpha canescens
Crown vetch	Coronilla varia	Cicer milkvetch	Astralagus cicer